Ornamental Horticulture
ISSN 2447-536X | WWW.ORNAMENTALHORTICULTURE.COM.BR
SBFPO Sociedade Brasileira de Floricultura e Plantas Ornamentais

Article

24-Epibrassinolide improves quality and antioxidant defense in cut *Dendrobium* 'Khao Sanan' flowers

O 24-epibrassinolídeo melhora a qualidade e a defesa antioxidante em flores cortadas de Dendrobium 'Khao Sanan'

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Abstract
Cut flower longevity is influenced by hormonal regulation, among which brassinosteroids (BRs) play a pivotal role in enhancing the postharvest quality of floricultural crops. However, the impact of BRs on cut *Dendrobium* orchids remains unexplored. This study aimed to investigate the effects of 24-epibrassinolide (EBL) on the physiological responses and senescence of cut *Dendrobium* 'Khao Sanan' flowers. Flower buds were soaked in 2.5, 5, 10, and 20 µM EBL solutions for 1 h, while distilled water served as the control treatment (0 µM). Subsequently, all flowers were placed in distilled water and maintained at 25 °C. Flower quality and opening scores were determined for morphological observations. Furthermore, physiological changes were evaluated by measuring ethylene production, respiration rate, hydrogen peroxide (H_2O_2) content, lipid peroxidation, and antioxidant enzyme activities and capacity during and at the end of storage. The results showed that flowers treated with 2.5 and 5 µM EBL exhibited substantially lower ethylene production, respiration rate, H_2O_2 content, and lipoxygenase (LOX) activity, along with reduced malondialdehyde (MDA) content. Moreover, flowers treated with 2.5 and 5 µM EBL displayed significantly higher catalase (CAT) and ascorbate peroxidase (APX) activities, as well as increased antioxidant capacity than the other treatments, whereas flowers treated with 20 µM EBL exhibited adverse responses, including accelerated senescence, increased oxidative damage, and reduced flower quality. This study indicates that low concentrations of EBL delayed flower senescence through enhanced antioxidant capacity, whereas high concentrations accelerated senescence. Therefore, cut *Dendrobium* 'Khao Sanan' orchids responded differentially to EBL in a concentration-dependent manner.
Keywords: Antioxidants, brassinosteroids, cut flower, flower senescence, lipid peroxidation.

Resumo
A longevidade de flores de corte é influenciada pela regulação hormonal, entre os quais os brassinosteroides (BRs) desempenham um papel fundamental na melhoria da qualidade pós-colheita de culturas florícolas. No entanto, o impacto dos BRs em orquídeas *Dendrobium* de corte permanece inexplorado. Este estudo teve como objetivo investigar os efeitos do 24-epibrassinolídeo (EBL) sobre as respostas fisiológicas e a senescência de flores de corte de *Dendrobium* 'Khao Sanan'. Os botões florais foram imersos em soluções de EBL nas concentrações de 2,5, 5, 10 e 20 µM durante 1 h, enquanto água destilada foi utilizada como tratamento controle (0 µM). Subsequentemente, todas as flores foram colocadas em água destilada e mantidas a 25 °C. A qualidade floral e os índices de abertura foram determinados por observações morfológicas. Além disso, alterações fisiológicas foram avaliadas pela mensuração da produção de etileno, taxa respiratória, conteúdo de peróxido de hidrogênio (H_2O_2), peroxidação lipídica e atividades e capacidade de enzimas antioxidantes durante e ao final do armazenamento. Os resultados mostraram que flores tratadas com 2,5 e 5 µM de EBL apresentaram produção de etileno, taxa respiratória, conteúdo de H_2O_2 e atividade da lipoxigenase (LOX) substancialmente menores, juntamente com redução do conteúdo de malondialdeído (MDA). Além disso, flores tratadas com 2,5 e 5 µM de EBL apresentaram atividades significativamente maiores de catalase (CAT) e ascorbato peroxidase (APX), bem como maior capacidade antioxidante do que os demais tratamentos, enquanto flores tratadas com 20 µM de EBL exibiram respostas adversas, incluindo aceleração da senescência, aumento dos danos oxidativos e redução da qualidade floral. Este estudo indica que baixas concentrações de EBL retardaram a senescência floral por meio do aumento da capacidade antioxidante, enquanto altas concentrações aceleraram a senescência. Portanto, orquídeas de corte *Dendrobium* 'Khao Sanan' responderam diferencialmente ao EBL de maneira dependente da concentração.
Palavras-chave: Antioxidantes, brassinosteroides, flor de corte, peroxidação lipídica, senescência floral.

Introduction

The deterioration of cut flowers due to senescence processes impacts their ornamental and economic value. The longevity of cut flowers is constrained by various factors, including environmental conditions, postharvest handling, and hormonal regulation (Costa et al., 2021; Janowska and Andrzejak, 2022), with particular emphasis on ethylene, which has been extensively studied for its role in flower senescence across many species (Dar et al., 2021). Besides ethylene, brassinosteroids (BRs) have been reported to be associated with a wide range of physiological and developmental processes, including senescence regulation and plant responses to biotic and abiotic stresses (Jiroutova et al., 2018). Recently, several studies have focused on the role of BRs as chemical elicitors for inducing defense against postharvest diseases (Song et al., 2022) and maintaining the quality of horticultural products, especially fruit and vegetable crops during postharvest storage (Gutiérrez-Villamil et al., 2023; Zhu et al., 2023). Treatments with 24-epibrassinolide (EBL), a highly bioactive, non-toxic and stable BR, have been reported to improve postharvest quality and extend longevity in many plant species (Hussain et al., 2020).

In broccoli, EBL treatment delayed yellowing and extended shelf life by maintaining chloroplast ultrastructure, reducing ethylene production (Cai et al., 2019) and enhancing superoxide dismutase (SOD), ascorbate peroxidase (APX), phenylalanine ammonia-lyase (PAL), and antioxidant capacity (Fang et al., 2021). Moreover, EBL improved antioxidant defense, reduced reactive oxygen species (ROS) accumulation and malondialdehyde (MDA) content, thereby delaying senescence in wucai (Yuan et al., 2021) and carambola fruit (Zhu et al., 2021).

However, there are only limited reports on the postharvest application of BRs in cut flowers. Recent evidence suggests that EBL application has the potential to maintain membrane integrity and enhance antioxidant enzyme activities in daylily flower buds during storage (Yao et al., 2017). Moreover, the balance of endogenous hormones such as gibberellic acid, zeatin riboside, and abscisic acid in daylily flower buds during storage was also maintained through EBL application. In addition, it was reported that EBL application enhanced or inhibited ethylene biosynthesis depending on its concentration in cut lisianthus flowers (Darvish et al., 2021). Furthermore, EBL could mitigate water deficit stress in cut *Dendrobium* orchid (Sukpitak et al., 2024).

***Corresponding author:** kanogwan.k@chula.ac.th | https://doi.org/10.1590/2447-536X.v32.e322970 | Editor: Lucas Cavalcante da Costa, Universidade Federal Rural da Amazônia, Brasil | Received: Jun 27, 2025 | Accepted: May 11, 2026 | Available online: June 15, 2026 |

Dendrobium orchids, an economically important cut flower crop, have been reported to be ethylene-sensitive flowers (Sukpitak and Seraypheap, 2023). Vase life of cut orchid flowers is mainly affected by external environmental factors and hormonal regulation, which accelerate flower senescence and decrease postharvest quality (Khunmuang et al., 2019). Hormonal signaling controlling petal senescence in orchids is not limited solely to ethylene but also involves other hormones, including BRs. While the role of BRs has been described in some cut flowers, there is no direct research evidence demonstrating their role in senescence of cut *Dendrobium* orchids, a major ornamental export product of Thailand. Thus, this study was conducted to investigate the effects of EBL application on postharvest physiological changes and flower senescence of *Dendrobium* 'Khao Sanan' orchid flowers. This work provides new insights into the role of BRs in cut orchid flower senescence and may contribute to future studies related to postharvest management in the cut flower industry.

Materials and methods

Plant material and treatments

Dendrobium 'Khao Sanan' inflorescences were harvested from a commercial farm in the central region of Thailand and delivered directly to the laboratory within 3 h. Healthy and uniform inflorescences with a similar number of floral buds and opened flowers were selected. The flower buds at the first position, counted from the uppermost opened flower, were carefully excised and placed in tubes containing distilled water until further treatment.

For the treatments, 24-epibrassinolide (EBL; purity ≥ 98%, MedchemExpress, NJ, USA) was first dissolved in 80% ethanol and then diluted with distilled water to final concentrations of 2.5, 5, 10, and 20 μM. All EBL solutions contained 0.05% (v v^{-1}) Tween 20 as a surfactant. The control group consisted of distilled water supplemented with the same concentration of ethanol and 0.05% (v v^{-1}) Tween 20, serving as a solvent control. Flower buds in each group were soaked in the respective EBL solutions for 1 h. After air-drying at room temperature for 10 min, the pedicels of individual flower buds were placed in distilled water and stored at 25 ± 1 °C and 80%-90% relative humidity under a 12 h photoperiod of fluorescent light at an intensity of approximately 10 μmol m^{-2} s^{-1}.

Evaluation of flower quality and bud opening

The flower quality score was used to determine morphological changes of the flowers during the vase life period. Visible senescence symptoms, such as venation, wilting, color change, and water-soaked appearance, served as indicators for scoring according to the methodology of Sukpitak and Seraypheap (2023), as follows: 4 = no signs of senescence symptoms; 3 = one symptom present; 2 = two symptoms present; and 1 = more than two symptoms present.

The flower opening score was assigned following Kongklom et al. (2018), as follows: 1 = no opening; 2 = sepal, petal, and lip separating; 3 = sepal, petal, and lip blooming; and 4 = fully open.

Measurement of respiration rate and ethylene production

Carbon dioxide (CO_2) production was measured to determine flower respiration rate by incubating flowers of known fresh weight in an airtight container for 2 h at 25 °C, after which a 1 mL headspace gas sample was withdrawn. After injection into a gas chromatograph (7890B, Agilent Technologies, Inc., USA), CO_2 production was calculated and expressed as respiration rate (mg CO_2 kg^{-1} s^{-1}). For ethylene production, values were expressed as ng kg^{-1} s^{-1}.

Assay of hydrogen peroxide content

With slight modifications, the method for measuring hydrogen peroxide (H_2O_2) in petals was followed as described by Junglee et al. (2014). Reaction mixture (1 mL) consists of 0.1 % (w v^{-1}) trichloroacetic acid (TCA) (0.25 mL), 50 mM potassium phosphate buffer, pH 7 (0.25 mL), and 1 M potassium iodide (0.5 mL) were used for one-step extraction of 0.1 g fine grounded petals. The supernatant was collected and incubated in the dark at room temperature for 20 min after centrifuging at 12,000 × g for 20 min at 4 ˚C. The production of H_2O_2 was determined by measuring the absorbance at 350 nm and then was calculated expressed on a fresh weight basis as mmol kg^{-1} using the known concentration of H_2O_2 standard curve.

Assay of malondialdehyde content

The malondialdehyde (MDA) content in petal tissue was assayed following the method of Ummarat et al. (2011). Petal tissue (0.3 g) was homogenized in 5% (w v^{-1}) TCA, and the homogenate was centrifuged at 12,000 × g for 20 min. The reaction mixture, consisting of supernatant (0.5 mL) and 15% (w v^{-1}) TCA containing 0.5% (w v^{-1}) thiobarbituric acid (TBA) (0.5 mL), was incubated at 95 °C for 30 min and then rapidly cooled on ice. Absorbance at 450, 532, and 600 nm was measured after centrifugation at 12,000 × g for 10 min. The MDA content was calculated and expressed on a fresh weight basis as mol kg^{-1} FW.

Determination of lipoxygenase activity

Lipoxygenase (LOX) was extracted and assayed following the methodology described by Sukpitak et al. (2024). An extraction buffer consisting of 100 mM potassium phosphate buffer (pH 8.0) with 1% (w v^{-1}) polyvinylpolypyrrolidone (PVPP) was used to extract 0.3 g of petal tissue. The homogenate was then centrifuged at 12,000 × g for 25 min at 4 °C. For the enzyme assay, 100 mM potassium phosphate buffer (pH 6.8), 10 mM sodium linoleic acid, and the supernatant were mixed to initiate the reaction. Absorbance was measured at 234 nm, and the specific activity of LOX was expressed as U kg^{-1} protein.

Determination of DPPH scavenging activity

Free radical 2,2-dipheynl-1-picrylhydrazyl (DPPH) scavenging activity was assayed using 0.2 g of petal tissue powder extracted in cold 80% ethanol. The supernatant was prepared by centrifugation at 10,000 × g at 4 ºC for 20 min. An ethanolic solution of 0.2 mM DPPH (180 μL) was added to 20 μl of petal extract and then incubated for 20 min at room temperature (Wantat et al., 2022). The reduction of DPPH was measured at 520 nm using 80% ethanol as the blank. For the control, 80% ethanol was used instead of the crude extract. The percentage of DPPH inhibition was calculated using the following equation:

$$\text{DPPH inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Determination of catalase and ascorbate peroxidase activities

Catalase (CAT) activity was assayed using the method of Song et al. (2014) with slight modifications. Approximately 0.3 g of petal tissue was homogenized in 50 mM potassium phosphate buffer (pH 7.0) containing 1% (w v^{-1}) polyvinylpolypyrrolidone (PVPP), 4 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. The homogenate was then centrifuged at 12,000 × g for 25 min at 4 °C. For the enzyme assay, the supernatant was added to a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0) and 10 mM H_2O_2 to initiate the reaction. The decrease in H_2O_2 absorbance was measured at 240 nm, and CAT activity was calculated and expressed as U kg^{-1} protein using the molar extinction coefficient (43.6 M^{-1} cm^{-1}).

Ascorbate peroxidase (APX) activity was extracted and assayed using 50 mM potassium phosphate buffer (pH 7.0) comprising 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ascorbic acid, and 1% (w v^{-1}) PVPP. After centrifugation, APX activity was assessed using a reaction mixture containing the collected supernatant, 50 mM potassium phosphate buffer (pH 7.0), 10 mM EDTA, 20 mM ascorbic acid, and 10 mM H_2O_2. Enzyme activity was calculated from the change in absorbance at 290 nm using the molar extinction coefficient (2.8 mM^{-1} cm^{-1}) and expressed as U kg^{-1} protein.

Statistical analysis

Flower quality and opening were evaluated with eight replicates (flowers) per treatment, while all other experiments were carried out in triplicate. Statistical analyses were performed using SPSS software version 28.0. Flower quality and opening scores were analyzed using the Kruskal–Wallis test, whereas all other data were analyzed using analysis of variance (ANOVA). Duncan's multiple range test was applied to compare treatment means and identify significant differences at $p < 0.05$.

Results

Flower quality and opening

Morphological changes in *Dendrobium* 'Khao Sanan' flowers over the vase period for each treatment are illustrated in Fig. 1. With increasing storage time, senescence symptoms became more pronounced in flowers treated with 20 µM EBL, as evidenced by venation and the onset of water-soaking symptoms on the petals by day 12. In contrast, flowers treated with 2.5 and 5 µM EBL maintained better visual quality, with fewer senescence symptoms compared to the control throughout storage. However, no obvious differences in visual appearance were observed between flowers treated with 10 µM EBL and the control group.

The quality of the flowers was assessed based on the degree of senescence symptoms. Flowers treated with 20 µM EBL showed a decrease in quality score by day 8, whereas a decline in the other treatments was observed only by day 12. On day 12, the highest quality scores were recorded in flowers treated with 2.5 and 5 µM EBL, while the lowest score was found in flowers treated with 20 µM EBL. There was no significant difference in quality between flowers treated with 10 µM EBL and the control group (Fig. 2A). These results indicate that low concentrations of EBL delayed flower senescence, whereas a high concentration (20 µM) accelerated senescence.

In terms of flower opening scores, no significant differences were observed among the treatments. These results suggest that EBL did not significantly influence flower opening under the conditions of this study (Fig. 2B).

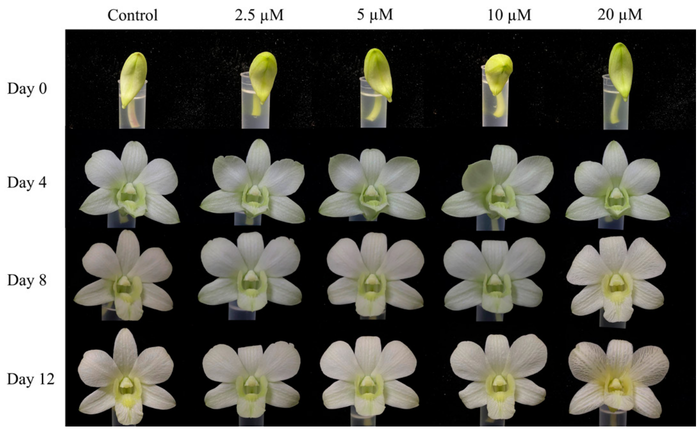

Fig. 1 Representative images showing flower opening and senescence progression in cut *Dendrobium* flowers treated with different EBL concentrations during storage. Visual differences were subtle among treatments during early storage, while accelerated senescence symptoms became evident in 20 µM EBL by Day 12, consistent with quantitative flower quality assessments.

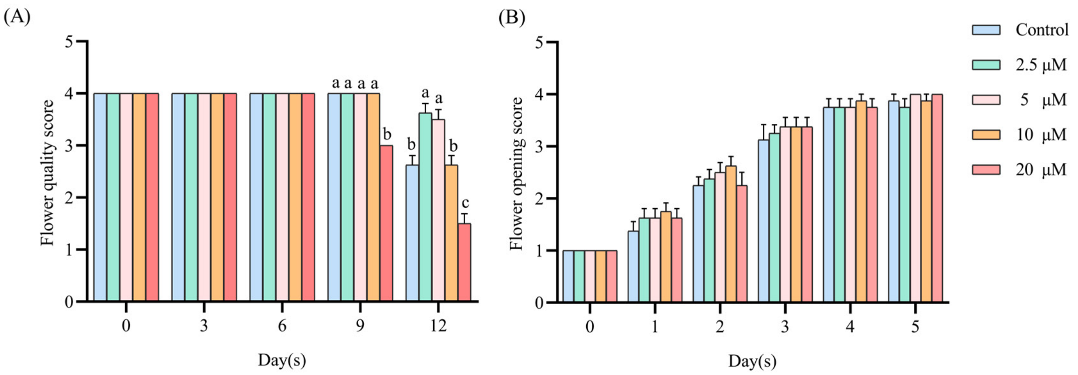

Fig. 2 Flower quality score **(A)** and flower opening score **(B)** of *Dendrobium* 'Khao Sanan' flower in different concentrations of EBL (control, 2.5, 5, 10, and 20 µM EBL) during vase periods. Data shown are the mean values ± S.E. (n=8) and different letters indicates the statistically significant difference among treatments ($p < 0.05$).

Ethylene production and respiration rate

The level of ethylene production exhibited a significant increase in flowers treated with 20 µM EBL (0.82 ng kg^{-1} s^{-1}), followed by those treated with 10 µM (0.72 ng kg^{-1} s^{-1}) and the control flowers (0.67 ng kg^{-1} s^{-1}), whereas flowers treated with 2.5 µM and 5 µM EBL showed lower ethylene content than the others (0.56 and 0.58 ng kg^{-1} s^{-1} respectively) on day 8 (Fig. 3A).

Flower respiration is shown in Fig. 3B. On day 8, flowers treated with 20 µM EBL displayed a significant increase in respiration rate compared to the other treatments, followed by a marked decrease on day 12. Conversely, the 2.5 µM EBL treatment tended to reduce the respiration rate by approximately 1.1-fold relative to the control on day 8, and this trend was maintained until day 12, although the reduction was not statistically significant.

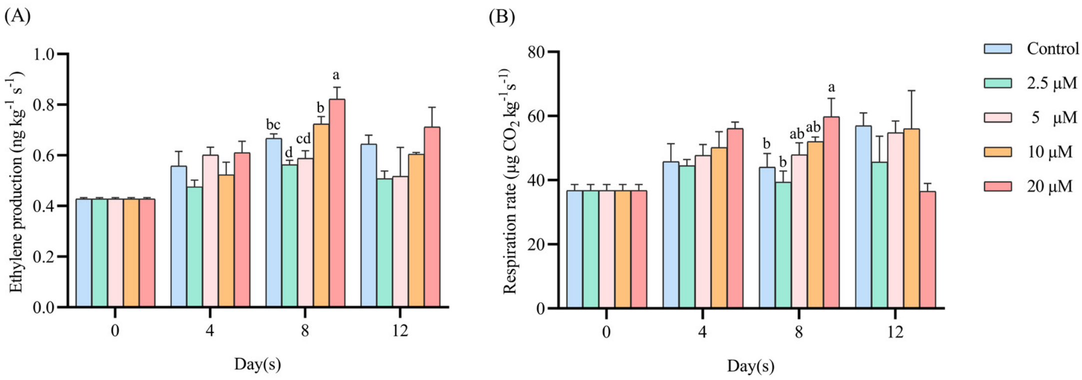

Fig. 3 Ethylene production (A) and respiration rate (B) of *Dendrobium* 'Khao Sanan' flower in different concentrations of EBL (control, 2.5, 5, 10, and 20 µM EBL) during vase periods. Data shown are the mean values ± S.E. (n=3) and different letters indicates the statistically significant difference among treatments ($p < 0.05$)

Hydrogen peroxide content

The changes in H_2O_2 content are shown in Fig. 4A. The application of 20 µM EBL led to a significant increase in H_2O_2 content (0.62 mmol kg^{-1}) on day 8 compared with the control (0.37 mmol kg^{-1}) and other EBL treatments (2.5 and 5 µM; 0.28 mmol kg^{-1}; 10 µM: 0.47 mmol kg^{-1}). Flowers treated with 10 µM EBL also exhibited higher H_2O_2 content than the control. In contrast, application of 2.5 and 5 µM EBL reduced H_2O_2 content by approximately 1.32-fold relative to the control

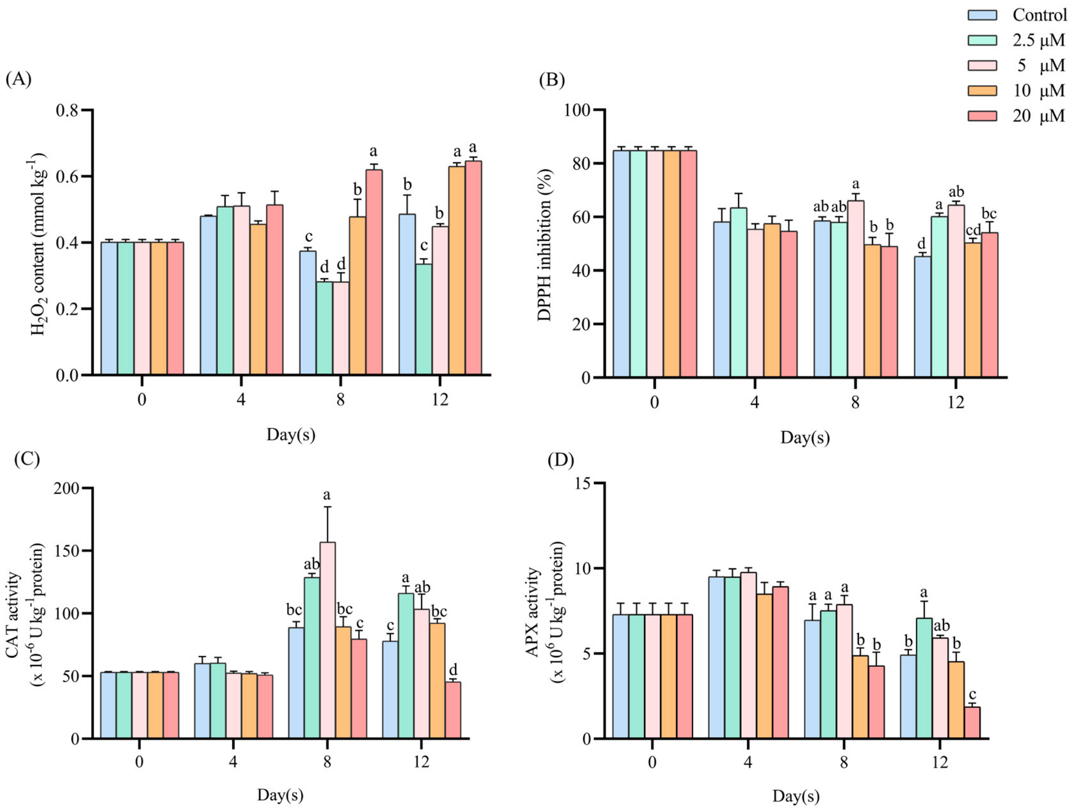

Fig. 4. H_2O_2 content (A), DPPH inhibition (B), CAT activity (C), and APX activity (D) of *Dendrobium* 'Khao Sanan' flower in different concentrations of EBL (control, 2.5, 5, 10, and 20µM EBL) during vase periods. Data shown are the mean values ± S.E. (n=3) and different letters indicates the statistically significant difference among treatments ($p < 0.05$).

DPPH scavenging

As shown in Fig. 4B, DPPH inhibition in control flowers gradually decreased during storage. On day 8, the percentage of DPPH inhibition in flowers treated with 5 µM EBL was higher than that of the other treatments, and it remained high until day 12, whereas flowers treated with 10 and 20 µM EBL exhibited the lowest inhibition. On day 12, the 2.5 and 5 µM EBL treatments resulted in approximately 1.4-fold greater DPPH inhibition compared with the control.

Antioxidant activity

CAT activity increased gradually in the control and in flowers treated with 10 and 20 µM EBL, reaching comparable levels by day 8. In contrast, CAT activity in flowers treated with 2.5 and 5 µM EBL was significantly greater than that in the other treatments. On day 12, flowers treated with 2.5 µM EBL maintained approximately 1.5-fold higher CAT activity than the control, while the lowest CAT activity was observed in flowers treated with 20 µM EBL. However, no significant differences in activity were observed between the 10 µM treatment and the control (Fig. 4C).

APX activity in flowers from all groups gradually decreased during storage. Flowers treated with 10 and 20 µM EBL exhibited a noticeable decrease in APX activity on day 8, whereas no significant differences were observed among the 2.5 µM, 5 µM EBL, and control groups. However, on day 12, flowers treated with 2.5 µM EBL showed the highest APX activity, approximately 1.4-fold higher than the control, while the 20 µM EBL treatment showed the lowest activity, approximately 2.6-fold lower than the control. There was no significant difference between flowers treated with 10 µM EBL and the control group (Fig. 4D).

Malondialdehyde content and lipoxygenase activity

As shown in Fig. 5, flowers treated with 20 µM EBL exhibited a dramatic increase in MDA content on day 8 and day 12 compared with the other treatments. Conversely, lower MDA content was observed in flowers treated with 2.5 and 5 µM EBL on day 8 (0.68 and 0.70 mol kg^{-1}, respectively), compared with the control (0.77 mol kg^{-1}). Flowers treated with 10 µM EBL showed a slightly higher MDA content than the control (Fig. 5A).

LOX activity exhibited a similar pattern to MDA accumulation (Fig. 5B). A significant increase in LOX activity was observed in the 20 µM EBL treatment on both days 8 and 12 compared with the other treatments. In contrast, the 2.5 and 5 µM EBL treatments decreased LOX activity by approximately 1.3-fold compared with the control. Although the 10 µM EBL treatment showed slightly higher LOX activity than the control, the difference was not significant.

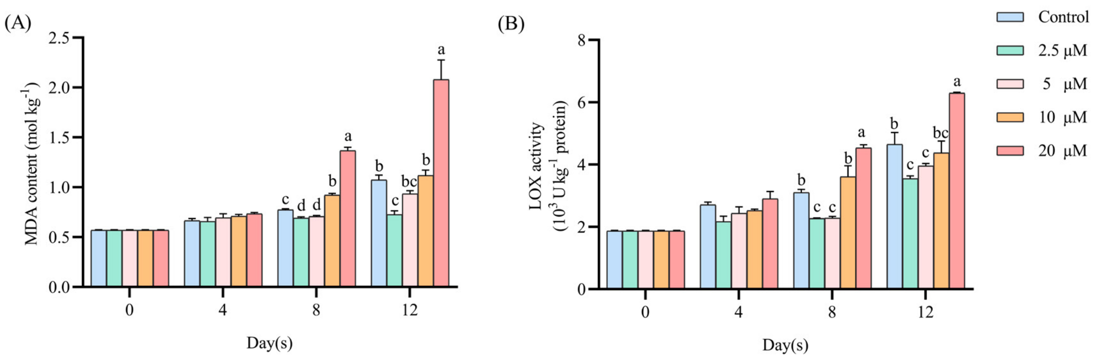

Fig. 5. MDA content (A) and LOX activity (B) of *Dendrobium* ‘Khao Sanan’ flower in different concentrations of EBL (control, 2.5, 5, 10, and 20 µM EBL) during vase periods. Data shown are the mean values ± S.E. (n=3) and different letters indicates the statistically significant difference among treatments ($p < 0.05$)

Discussion

BRs are plant steroidal hormones that play a crucial role in plant growth and development (Jiroutova et al., 2018). Over the past decade, the role of BRs in postharvest horticultural products has been increasingly recognized for improving quality and postharvest life (He et al., 2018; Islam et al., 2022). In the present study, EBL application regulated flower senescence during storage. However, contrasting responses were observed between low and high EBL concentrations, indicating a concentration-dependent effect. Compared with the control, flowers treated with 2.5 and 5 µM EBL exhibited delayed senescence symptoms, whereas those treated with 20 µM EBL showed accelerated senescence. Flowers treated with 10 µM showed a similar appearance to those in the control group. Based on morphological changes, flower quality scores were positively associated with senescence symptoms. Higher quality scores were observed in the 2.5 and 5 µM EBL treatments, whereas the lowest score was recorded in the 20 µM EBL treatment. These findings are consistent with previous reports showing that different concentrations of BRs can exert distinct effects on senescence regulation (Cai et al., 2019; Darvish et al., 2021). This may reflect a concentration-dependent response, in which low EBL concentrations stimulate protective physiological responses, whereas supra-optimal concentrations may accelerate senescence. Therefore, our results suggest that EBL regulates flower senescence in cut *Dendrobium* ‘Khao Sanan’ orchids in a concentration-dependent manner, with low concentrations delaying senescence and a high concentration (20 µM) accelerating senescence.

It has been well documented that ethylene plays a key role in driving senescence progression in climacteric flowers (Dar et al., 2021). In this study, treatment with 20 µM EBL significantly increased ethylene production, hastening the onset of visible senescence symptoms. Conversely, treatments with 2.5 and 5 µM EBL reduced ethylene production to levels lower than the control. Likewise, in cut lisianthus flowers, low concentrations of EBL delayed senescence and extended vase life by inhibiting the activity of ACC oxidase (ACO), an enzyme involved in ethylene biosynthesis, thereby reducing ethylene production (Darvish et al., 2021). Furthermore, treatment of broccoli with a high concentration of EBL (20 µM) upregulated genes involved in ethylene biosynthesis, resulting in a substantial increase in ethylene production, whereas lower EBL concentrations showed opposite effects (Cai et al., 2019). Research in tomato (Zhu et al., 2015) and persimmon fruit (He et al., 2018) revealed that BR application induced ripening by increasing ethylene production and respiration rates. In contrast, recent research in pear fruit showed that BR treatment suppressed ethylene production and delayed ripening through regulation of BR-ethylene crosstalk. Specifically, BR-activated BZR1, a key transcription factor in the BR signaling pathway, inhibited ACO1 activity and downregulated expression of ACO1 and ACS1, major ethylene biosynthesis genes (Ji et al., 2021). In addition, low concentrations of BRs in Arabidopsis suppressed ethylene production and upregulated BES1/BZR1, whereas elevated BR levels reduced BES1/BZR1 expression, thereby promoting ethylene biosynthesis (Lv et al., 2018). Therefore, the induction or suppression of ethylene production by BRs appears to be concentration dependent.

It has been reported that an increase in respiration rate is accompanied by increased ethylene production in climacteric flowers and fruit during senescence or ripening (He et al., 2018; Wongjunta et al., 2021). Exogenous application of EBL has been shown to reduce respiration rate, contributing to the maintenance of quality and delayed senescence in fresh daylily flower buds during storage (Yao et al., 2017). Additionally, EBL treatment decreased respiration rate and prolonged the shelf life of carambola fruit by altering the activity of key enzymes in the respiratory pathway (Zhu et al., 2021). In the present study, respiration rate showed a pattern consistent with ethylene production. Flowers treated with 20 µM EBL exhibited the highest respiration rate, whereas application of 2.5 µM EBL tended to reduce respiration in cut flowers. These results suggest that low concentrations of EBL may help maintain flower quality, partly through reduced respiratory activity and delayed senescence.

Visible signs of petal senescence include color changes, venation, and wilting (Kirasak et al., 2023), which were likewise observed in this study by day 12 in orchid flowers treated with a high concentration (20 µM) of EBL. Before these visual symptoms become evident, several physiological and biochemical changes occur in petal cells, including loss of membrane integrity and degradation of proteins, lipids, and nucleic acids, often associated with excessive accumulation of ROS, such as superoxide (O_2^-), hydroxyl radical (OH), and H_2O_2 (Rogers, 2012; Zou et al., 2014). For example, high production of ethylene and O_2^- during senescence of sweet osmanthus flowers was positively associated with lipid peroxidation and

DNA fragmentation, leading to cellular damage (Zou et al., 2014). Excessive ROS accumulation, particularly H_2O_2, can induce oxidative damage and membrane lipid peroxidation, resulting in loss of membrane integrity and cellular deterioration (Rogers, 2012). In our experiment, a high concentration of EBL (20 μM) led to increased H_2O_2 accumulation, accompanied by elevated MDA content, indicating enhanced lipid peroxidation. Conversely, treatment with low concentrations of EBL (2.5 and 5 μM) suppressed H_2O_2 accumulation and reduced MDA content. The 10 μM EBL treatment showed slightly higher levels than the control, although the differences were not significant. These results suggest that low EBL concentrations may alleviate oxidative damage, whereas a supra-optimal concentration promotes ROS accumulation associated with accelerated senescence.

The activity of the LOX enzyme, responsible for lipid breakdown, showed a pattern consistent with MDA levels in our study. Likewise, reductions in H_2O_2 and MDA content following EBL treatment during storage have also been reported in various postharvest horticultural products, including wucai (Yuan et al., 2021), daylily flower buds (Yao et al., 2017), carambola (Zhu et al., 2021), and blueberry (Min et al., 2022). Additionally, EBL has been reported to significantly decrease LOX activity, resulting in lower MDA accumulation, thereby suggesting improved membrane stability (Gao et al., 2017; Islam et al., 2022). Together, these findings suggest that EBL application, particularly at low concentrations, may alleviate lipid peroxidation and help preserve membrane integrity in *Dendrobium* 'Khao Sanan' flowers.

At the onset of flower senescence, antioxidant capacity may initially increase as a protective response; however, imbalance between ROS generation and scavenging systems ultimately leads to oxidative damage (Haq et al., 2024). Therefore, plants possess well-defined antioxidant defense mechanisms that help neutralize excess ROS within their cells. The results of our study demonstrated that treatment with low concentrations of EBL maintained higher antioxidant capacity, including increased activities of CAT and APX, two key antioxidant enzymes involved in H_2O_2 detoxification in plant cells (Mishra et al., 2023). These findings are consistent with previous studies showing that enhancement of antioxidant defenses is an important mechanism underlying delayed senescence. EBL treatment has been reported to significantly increase the activities of SOD, peroxidase (POD), CAT, and APX, thereby alleviating oxidative damage in daylily flowers (Yao et al., 2017). In addition, EBL treatment has been shown to increase total antioxidant capacity and the activity of ascorbate (AsA) -glutathione (GSH) cycle-related enzymes during storage, helping maintain redox homeostasis and extend the postharvest life of wucai (Yuan et al., 2021). Furthermore, EBL treatment delayed ripening in blueberries by upregulating the activities of SOD, CAT, and PAL (Min et al., 2022). In our experiment, increased CAT and APX activities induced by low EBL concentrations were associated with reduced H_2O_2 accumulation, which may have contributed to lower oxidative damage in the flowers. Therefore, EBL treatment may delay visible senescence symptoms in cut *Dendrobium* 'Khao Sanan' flowers by enhancing antioxidant defense systems, thereby maintaining ROS homeostasis and reducing lipid peroxidation.

Conclusions

In summary, the effect of EBL on flower senescence in cut *Dendrobium* 'Khao Sanan' showed a concentration-dependent response. A high concentration (20 μM) of EBL accelerated flower senescence, as evidenced by increased ethylene production, respiration rate, H_2O_2 accumulation, MDA levels, and LOX activity. Conversely, low concentrations of EBL (2.5 and 5 μM) delayed petal senescence by decreasing ethylene production, respiration rate, H_2O_2 accumulation, MDA levels, and LOX activity, while enhancing antioxidant defenses, including CAT and APX activities and overall antioxidant capacity. Additionally, flowers treated with 10 μM EBL showed no significant difference in quality compared with the control. Taken together, these findings suggest that low-concentration EBL treatment may help preserve postharvest quality in cut *Dendrobium* 'Khao Sanan' flowers, partly through enhanced antioxidant defense and reduced oxidative damage.

Acknowledgments

The authors gratefully acknowledge Mr. Year Bune Seraypheap for his valuable support in revising the manuscript and improving the language.

Author Contribution

CS: Conceptualization, Investigation, Methodology, Formal Analysis, Writing - Original Draft. **KS:** Conceptualization, Methodology, Supervision, Writing - Review & Editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

All the research data is contained in the manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

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